

Predicting Liver-Related Outcomes in People With Nonalcoholic Fatty Liver Disease: The Prognostic Value of Noninvasive Fibrosis Tests

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It remains unclear whether screening for advanced fibrosis in the community can identify the subgroup of people with nonalcoholic fatty liver disease (NAFLD) at higher risk for development of liver-related complications. We aimed to determine the prognostic value of baseline noninvasive fibrosis tests for predicting liver-related outcomes and mortality in patients with NAFLD from type 2 diabetes (T2D) clinics or primary care. Patients (n = 243) who were screened for NAFLD with advanced fibrosis by using NAFLD fibrosis score (NFS), fibrosis 4 score (FIB-4), enhanced liver fibrosis (ELF) test, and liver stiffness measurements (LSMs) were followed up for clinical outcomes by review of electronic medical records. During a median follow-up of 50 months, decompensated liver disease or primary liver cancer occurred in 6 of 35 (17.1%) patients with baseline LSM > 13 kPa, 1 of 17 (5.9%) patients with LSM 9.5-13 kPa, and in no patients with LSM < 9.5 kPa. No patient with low-risk NFS developed liver decompensation or liver-related mortality. Following repeat NFSs at the end of follow-up, all patients with a liver-related complication were in the high-risk NFS category. Patients who developed liver-related complications were also more likely to have baseline high-risk FIB-4 scores or ELF test ≥ 9.8 compared to patients who did not develop liver outcomes. *Conclusion:* Liver fibrosis risk stratification in non-hepatology settings can identify the subset of patients at risk of liver-related complications. Although the rate of development of a decompensation event or hepatocellular carcinoma was low (2.1% per year) in our patients with compensated cirrhosis (LSM > 13 kPa), these events are projected to lead to a substantial increase in NAFLD-related disease burden over the next decade due to the high prevalence of NAFLD in people with obesity and T2D. (*Hepatology Communications* 2022;6:728-739).

Nonalcoholic fatty liver disease (NAFLD) is an increasingly important chronic liver disease due to its high prevalence in up to 80% of people with obesity and in 40%-70% of people with type 2 diabetes (T2D).^(1,2) Although most people with NAFLD do not manifest clinically significant liver disease, 5%-10% develop advanced fibrosis and are at risk of developing complications of cirrhosis, including

Abbreviations: BMI, body mass index; CI, confidence interval; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; HR, hazard ratio; IQR, interquartile range; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; NFS, nonalcoholic fatty liver disease fibrosis score; T2D, type 2 diabetes.

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hepatocellular carcinoma (HCC), over a period of 10–20 years.⁽³⁾ A central issue is the ability to detect the patients at greatest risk of liver complications among the vast number of affected individuals. The key prognostic marker for adverse liver outcomes is the presence of advanced fibrosis (including bridging, stage 3) and cirrhosis (stage 4),⁽³⁾ which can be estimated using noninvasive biomarkers, such as serum fibrosis scores or ultrasound-based measurement of liver stiffness or elasticity.⁽⁴⁾ However, despite an increasing liver-related burden of NAFLD,⁽⁵⁾ cardiovascular disease and extrahepatic malignancy remain the leading causes of death in this patient group.⁽³⁾

Due to the high prevalence of NAFLD in primary care and in people with cardiometabolic risk factors, several local health districts and specialty networks are exploring the role of integrated referral pathways and management plans for NAFLD.^(6–11) Once a diagnosis of NAFLD is made, assessment of fibrosis severity is important in order to guide decisions about patient referral and follow-up. Although there are currently no guidelines for NAFLD assessment in the community in Australia, “expert opinion” recommends using simple fibrosis scores (NAFLD fibrosis score [NFS] or fibrosis-4 [FIB-4] test) as a first step to identify individuals at low risk of advanced fibrosis who can be managed in primary care. People with indeterminate or high-risk simple scores need additional assessment with second-line fibrosis tests (such as ultrasound elastography or serum enhanced liver fibrosis [ELF] test), and those at increased risk of significant liver disease require hepato-logy referral for confirmation of advanced fibrosis.⁽¹²⁾

It remains unclear, however, whether screening for advanced fibrosis in the community can identify the subgroup of people with NAFLD at higher risk for mortality, development of liver-related complications, or adverse cardiovascular or malignancy-related events. In addition, it is not clear whether the cut-off values for noninvasive biomarkers used to estimate advanced fibrosis and cirrhosis are optimal for the prediction of clinical outcomes. In this study, we sought to determine the prognostic value of baseline individual simple fibrosis scores, liver stiffness measurement (LSM), or serum ELF test for predicting liver- and non-liver-related outcomes as well as mortality in patients from an endocrine clinic or primary care facility who underwent screening for NAFLD with clinically significant fibrosis.⁽¹³⁾ In addition, we examined the prognostic value of longitudinal changes in the NFS and FIB-4 scores for predicting clinical outcomes.

Patients and Methods

PATIENTS AND BASELINE CLINICAL DATA

Patients recruited to our prior study assessing the prevalence of NAFLD with clinically significant fibrosis in T2D clinics and at-risk populations in primary care⁽¹³⁾ were followed up for clinical outcomes by review of electronic medical records. Informed written consent was obtained from patients, and the protocol was approved by the

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Metro South Health and University of Queensland human research ethics committees (HREC/15/QPAH/301; UQ2015001047). In the prior study, medical history was obtained during consultation using a structured questionnaire, including socio-demographic characteristics, previously diagnosed liver disease, medical conditions, and use of medications. Alcohol intake was assessed using a standardized questionnaire and the Alcohol Use Disorders Identification Test.⁽¹⁴⁾ Patients underwent a clinical assessment that included anthropometric measurements, laboratory tests (routine biochemical, hematologic, and serologic assays and ELF test), and liver ultrasound.

Transient elastography was performed after a 3-hour fast by using FibroScan technology (Echosens, Paris, France) with the standard M or XL probes in line with the manufacturer's instructions. Examinations were performed by a trained clinical nurse (L.U.H.; >400 LSMs performed) and reviewed by a hepatologist with extensive FibroScan experience (>2,000 LSMs performed). Recommended standard FibroScan operating procedures were followed along with adherence to criteria for the definition of reliable LSMs as follows: minimum of 10 valid measurements with a success rate of $\geq 60\%$ and interquartile range (IQR) $\leq 30\%$ of the final result. The XL probe was used when the skin-capsule depth was ≥ 2.5 cm. For the purposes of this study, we used LSM cut-off values of 8.0 kPa for clinically significant fibrosis, ≥ 9.5 kPa for advanced fibrosis, and >13 kPa to indicate cirrhosis; the same cut-off value was used for both probes.⁽¹³⁾

Fibrosis risk stratification using NFS and FIB-4 scores was obtained using readily available online calculators hosted by MDCalc (<https://www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis#evidence>; <https://www.mdcalc.com/nafl-d-non-alcoholic-fatty-liver-disease-fibrosis-score#evidence>). The online FIB-4 calculator applied an age-adjusted lower cut-off (<2.0) to exclude advanced fibrosis in patients aged ≥ 65 years,⁽¹⁵⁾ whereas the NFS calculator did not.

FOLLOW-UP CLINICAL DATA

All patients recruited to our prior study had at least one entry in the hospital electronic medical record. Each patient's electronic medical record was

reviewed to determine the date of the last recorded medical contact and to collect clinical data, including date and type of liver-related events, cardiovascular events, malignancy, most recent anthropometric measurements, laboratory tests, imaging, and FibroScan results (if available). The electronic medical record provided access to each patient's public hospital admission discharge summaries, procedures, pathology results, and imaging reports throughout the state. Private pathology services were also accessed if hospital pathology results were not available within the last 2 months. If the last recorded event was >12 months before the date of ascertainment of follow-up data and/or information in the patient's electronic medical record was deemed to be insufficient to capture all events of interest, the patient's general practitioner was contacted to provide further information. We did not contact private hospitals. A flow diagram for ascertainment of follow-up data is available in Supporting Fig. S1. Clinical data were collected by two physicians (E.E.P. and A.L.J.) and a research nurse (L.U.H.), and a sample of 20 patients was cross-checked for concordance of data collection.

OUTCOME MEASUREMENTS

The primary outcome measures were (i) the development of a liver-related complication, including a) liver decompensation with ascites (confirmed by paracentesis or abdominal imaging), hepatic encephalopathy (defined clinically), or variceal hemorrhage (confirmed by endoscopy); b) primary liver cancer (HCC or cholangiocarcinoma, diagnosed by imaging methods or by histology); and c) portal hypertension (splenomegaly, varices, or portosystemic collaterals); and (ii) mortality (death of any cause). Secondary outcome measures were a change in fibrosis risk category after recruitment, development of malignancy (excluding primary liver cancer), and cardiovascular events.

DATA ANALYSIS

Continuous variables were described by mean and SD or, if appropriate, median and IQR. Categorical variables were presented as counts and percentages. For nonpaired analyses, significant differences between groups were assessed by *t* test (continuous

variables) and chi-squared test (categorical variables; Fisher's exact test was used for sparse tables). For paired analyses, significant differences between groups were assessed by Wilcoxon signed-rank test, exact McNemar's test, and Brooker's test. Kaplan-Meier curves (log-rank statistic) were generated for time to liver-related event stratified by a) LSM < 8 kPa, LSM 8 to <9.5 kPa, ≥ 9.5 to 13 kPa, and >13 kPa⁽¹³⁾; b) low, indeterminate, or high-risk NFS; c) low, indeterminate, or high-risk FIB-4 score; and d) ELF score < 9.8, ELF score ≥ 9.8 , ELF score ≥ 11.3 . All cases were followed up until date of liver-related event, date of death, or date of last medical contact, whichever came first. Multivariable Cox regression analysis reported in terms of hazard ratios (HRs) with associated 95% confidence intervals (CIs) was used to assess differences by LSM, NFS, FIB-4, or ELF scores as a continuous measure with respect to liver-related event, mortality, non-liver malignancy, and cardiovascular events. The models were adjusted for age and known cirrhosis before recruitment. The proportional hazards assumption was examined by the Schoenfeld residuals in STATA version 16.1 (StataCorp, College Station, TX).

Results

PATIENT COHORT AND CLINICAL OUTCOMES

The study cohort comprised 243 of 252 patients with NAFLD recruited from T2D clinics and at-risk populations in primary care between October 2015 and August 2017.⁽¹³⁾ Patients in the cohort had at least 12 months follow-up or had died within 12 months of follow-up. One patient withdrew consent for the study, and 8 patients had <12 months follow-up. The median age of the 8 excluded patients was significantly lower than the rest of the cohort ($P = 0.028$), and fewer excluded patients were recruited from the Diabetes Clinic ($P = 0.047$; Supporting Table S1). Demographic and clinical characteristics at the time of recruitment and at the time of last follow-up are summarized in Table 1. LSM > 13 kPa, consistent with cirrhosis, was present in 35 patients (14.4%) at recruitment and was a new diagnosis in 30 (85.7%) of these 35 patients. Another patient with known cirrhosis at study

recruitment had an LSM that did not meet quality criteria. Characteristics of the 6 patients with known cirrhosis at the study recruitment are summarized in Supporting Fig. S2.

The median follow-up time from recruitment to the last medical contact recorded in the hospital/primary care electronic medical record or date of death was 4.18 years (range, 0.52-5.62 years; IQR, 3.71-4.50 years). At follow-up, there was a small decrease in the proportion of patients with class 2 and 3 obesity ($P < 0.001$) and a decrease in median serum alanine aminotransferase level ($P < 0.001$) and serum albumin level ($P < 0.001$). The median NFS at follow-up was significantly higher ($P < 0.001$), but there was no significant difference between NFS categories ($P = 0.197$). The median FIB-4 at follow-up was significantly higher ($P < 0.001$), with significant difference according to FIB-4 categories ($P < 0.001$). A higher proportion of patients with LSM ≥ 9.5 kPa was seen in a hepatology clinic in the past 12 months compared to patients with LSM < 9.5 kPa (61.5% vs. 14.4%, respectively, $P < 0.001$).

Seven patients (2.9%) experienced at least one liver decompensation event (ascites, $n = 2$; variceal bleeding, $n = 1$; hepatic encephalopathy, $n = 1$) or primary liver cancer (HCC, $n = 3$; cholangiocarcinoma, $n = 1$) during the follow-up period. Of these 7 patients, 6 had evidence of portal hypertension (endoscopic or radiologic evidence of varices, $n = 4$; other radiologic features of portal hypertension, $n = 2$). An additional 12 patients developed portal hypertension (endoscopic or radiologic evidence of varices, $n = 9$; other radiologic features of portal hypertension, $n = 3$). The baseline characteristics of patients with ($n = 19$) and without ($n = 224$) a liver-related complication are summarized in Table 2. Patients who developed a liver-related complication had higher baseline body mass index (BMI) ($P = 0.029$) and higher LSM, ELF, and simple fibrosis scores (all $P < 0.001$). All patients with a liver-related complication had T2D, and 6 (31.6%) patients were known to have cirrhosis at recruitment.

Overall, 35 patients (14.4%) experienced a non-liver malignancy (excluding nonmelanoma skin cancers; melanoma, $n = 8$; breast, $n = 7$; cervical, $n = 4$; prostate, $n = 3$; colorectal, $n = 3$; pancreatic, $n = 2$; chronic lymphocytic leukemia, $n = 1$; lung, $n = 1$; lymphoma, $n = 1$; esophageal, $n = 1$; renal cell, $n = 1$;

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS (N = 243*) AT RECRUITMENT AND LAST MEDICAL REVIEW

	At Recruitment		At Follow-Up		PValue
Age (years), median (IQR)	59	(50.0-67.0)			
Recruited from diabetes clinic, n (%)	102	42.0%			
Male sex, n (%)	129	53.1%			
Caucasian ethnicity, n (%)	192	79.0%			
Type 2 diabetes diagnosis, n (%)	202	83.1%	205	84.4%	0.250 [‡]
Extrahepatic malignancy, [†] n (%)	25	10.3%	35	14.4%	0.002 [‡]
Cardiovascular disease, n (%)	55	22.6%	97	39.9%	<0.001 [‡]
BMI (kg/m ²), median (IQR)	33.5	(29.8-39.3)	32.8	(28.5-38.3)	<0.001 [§]
BMI category, n (%)					<0.001 [§]
Normal weight	10	4.5%	17	7.6%	
Overweight	48	21.6%	61	27.5%	
Class I obesity	64	28.8%	60	27.0%	
Class II obesity	50	22.5%	42	18.9%	
>Class III obesity	50	22.5%	42	18.9%	
Known cirrhosis at recruitment [¶]	6	(2.5%)	-	-	-
Serum liver enzymes, median (IQR)					
ALT (IU/mL)	33	(22-52)	27	(20-40)	<0.001 [§]
AST (IU/mL)	23	(17- 35)	23	(18-32)	0.984 [§]
GGT (IU/mL)	33	(20-60)	34	(21-57)	0.416 [§]
Platelet (×10 ⁹)	241	(204-290)	243	(195-288)	0.222 [§]
Albumin (g/L)	41	(39-43)	40	(37-43)	<0.001 [§]
LSM (kPa), median (IQR) [#]	6.1	(4.8-9.1)			
LSM (kPa) categories, n (%)					
<8	149	(61.3%)			
8-9.4	20	(8.2%)			
9.5-13	17	(7.0%)			
>13	35	(14.4%)			
unreliable	22	(9.1%)			
NFS, median (IQR) ^{**}	-0.23	(-1.41 to 0.48)	0.02	(-1.06 to 0.90)	<0.001 [§]
NFS, n (%) ^{**}					
Low	39	17.6%	41	18.5%	0.197 [§]
Indeterminate	124	55.9%	110	49.6%	
High	59	26.5%	71	32.0%	
FIB-4 score, median (IQR) ^{††}	0.99	(0.69-1.41)	1.21	(0.85-1.61)	<0.001 [§]
FIB-4, n (%) ^{††}					
Low	143	59.1%	184	76.0%	<0.001 [§]
Indeterminate	89	36.8%	45	18.6%	
High	10	4.1%	13	5.4%	

*9 patients excluded from original study cohort⁽¹³⁾ (n = 8, follow-up <12 months; n = 1, withdrawal of study consent).

[†]excluding nonmelanoma skin cancers.

[‡]exact McNemar's test.

[§]Wilcoxon signed-rank test.

^{||}21 patients without a BMI completed within 12 months were excluded.

[¶]LSM ≥13 kPa, n = 5; LSM did not meet quality criteria, n = 1.

[#]22 patients with unreliable LSM results were excluded.

^{**}n = 222 (n = 20 excluded as BMI > 1 year from corresponding blood test results; n = 1 excluded with myelodysplastic syndrome).

^{††}n = 242 (n = 1 patient excluded due to myelodysplastic syndrome).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

TABLE 2. BASELINE CHARACTERISTICS OF PATIENTS WITH AND WITHOUT A LIVER-RELATED COMPLICATION

Baseline Variables	No Liver-Related Complication	Liver-Related Complication	P Value
	n = 224	n = 19	
Age (years), median (IQR)	59 (50-66.5)	62 (56-70)	0.23*
Male sex, n (%)	119 (53.1%)	10 (52.6%)	0.97†
Historical alcohol use, n (%)			0.78‡
none	63 (28.1%)	4 (21.1%)	
<20 g/day	107 (47.8%)	11 (57.9%)	
>20 g/day	54 (24.1%)	4 (21.1%)	
Smoking status, n (%)			0.68‡
Never smoker	120 (53.6%)	9 (47.4%)	
Ex-smoker	81 (36.2%)	9 (47.4%)	
Current smoker	23 (10.3%)	1 (5.3%)	
BMI (kg/m ²), median (IQR)	32.9 (29.3-38.23)	37.6 (31.3-45.3)	0.029*
BMI categories, n (%)			0.047‡
Normal weight	10 (4.5%)	1 (5.3%)	
Overweight	56 (25.0%)	1 (5.3%)	
Class I obesity	66 (29.5%)	5 (26.3%)	
Class II obesity	49 (21.9%)	3 (15.8%)	
>Class III obesity	43 (19.2%)	9 (47.4%)	
Type 2 diabetes, n (%)	183 (81.7%)	19 (100.0%)	0.041‡
LSM (kPa), median (IQR) [§]	6.0 (4.7-8.2)	17.1 (14.1-34.3)	<0.001*
LSM (kPa) categories, n (%)			<0.001‡
<8	149 (66.5%)	0	
8-9.4	20 (8.9%)	0	
9.5-13	14 (6.3%)	3 (15.8%)	
>13	21 (9.4%)	14 (73.7%)	
unreliable	20 (8.9%)	2 (10.5%)	
Known cirrhosis at recruitment, n (%)	0	6 (31.6%)	<0.001‡
ELF score, median (IQR)	9.18 (8.56-9.81)	10.74 (9.45-11.4)	<0.001*
ELF categories, n (%)			<0.001‡
<9.8	168 (75.0%)	5 (26.3%)	
9.8 to <11.3	51 (22.8%)	9 (47.4%)	
≥11.3	5 (2.2%)	5 (26.3%)	
Baseline NFS, median (IQR)	-0.54 (-1.52 to 0.31)	0.49 (0.03-2.19)	<0.001*
Baseline NFS categories, n (%)			<0.001‡
Low	45 (20.1%)	0	
Indeterminate	130 (58.0%)	7 (36.8%)	
High	49 (21.9%)	12 (63.2%)	
Baseline FIB-4, median (IQR)	0.97 (0.67-1.35)	1.61 (1.21-2.89)	<0.001*
Baseline FIB-4 categories, n (%)			<0.001‡
Low	138 (61.6%)	5 (26.3%)	
Indeterminate	82 (36.6%)	7 (36.8%)	
High	4 (1.8%)	7 (36.8%)	

*Wilcoxon signed-rank test.

†Pearson's chi-squared test.

‡Fisher's exact *t* test.

§n = 221 patients with LSM meeting quality criteria.

small bowel, $n = 1$; supraglottic plasmacytoma, $n = 1$; uterine, $n = 1$), with 10 of these malignancies developing during follow-up (Table 1). A total of 97 patients (39.9%) had a diagnosis of cardiovascular disease. In 42 of these patients, the diagnosis of cardiovascular disease was first noted after recruitment.

During the follow-up period, 17 patients (7%) died, with causes of death listed in Supporting Table S2. Causes of death included cardiovascular ($n = 4$), extrahepatic malignancy ($n = 3$), liver related ($n = 3$), infection related ($n = 2$), unknown ($n = 1$), and other ($n = 4$).

RELATIONSHIP BETWEEN BASELINE SCORES AND LIVER-RELATED COMPLICATIONS

Baseline LSM

LSM met quality criteria in 221 (90.9%) patients and required use of the XL probe in 76.9% of cases. Median LSM was 6.1 kPa (IQR, 4.8-9.1) with a range from 2.5 to 63.9 kPa. The proportion of patients with LSM ≥ 8.0 kPa (consistent with clinically significant fibrosis), ≥ 9.5 kPa (consistent with advanced fibrosis), and > 13 kPa (consistent with cirrhosis), according to development of liver-related outcomes, is summarized in Table 2. Of the 19 patients with a liver-related complication, baseline LSM was > 13 kPa in 14 patients, 9.5-13 kPa in 3, and did not meet quality criteria in 2 patients. Of the 7 patients who experienced a liver decompensation event and/or diagnosis of primary liver cancer, 6 had baseline LSM > 13 kPa and 1 had baseline LSM 9.5-13 kPa.

After adjustment for age and known cirrhosis before recruitment, patients with LSM > 13 kPa were 27.4 times more likely to have a liver-related event than patients with LSM < 13 kPa (95% CI, 7.86-95.50; $P < 0.001$). For every 1 unit increase in LSM, there was a 7% increase in the hazard of having a liver-related event (HR, 1.07; 95% CI, 1.05-1.10; $P < 0.001$), with a similar result following adjustment for age and known cirrhosis status (HR, 1.07; 95% CI, 1.04-1.10; $P < 0.001$).

In patients with compensated cirrhosis (LSM > 13 kPa), the rate of development of a decompensation event was 2.1% per year and the rate of development of HCC was 2.1% per year (annual incidence of HCC, 20.8 per 1,000 person-years).

Baseline Simple Fibrosis Scores

The proportion of patients with low, indeterminate, or high baseline NFS and FIB-4 scores, according to development of liver-related complications, is summarized in Table 2. No patient with a liver-related complication had a low-risk baseline NFS score. In contrast, a low FIB-4 score was present at baseline in 5 patients.

Baseline ELF Score

In Australia, the ELF test is marketed with a recommended cut-off value of ≥ 9.8 for "severe fibrosis."⁽¹⁶⁾ Standardized thresholds for the ELF test for the detection of advanced fibrosis (9.8) and cirrhosis (11.3) have been described and are recommended for use in the interpretation of test results.⁽¹⁷⁾ The proportion of patients with ELF < 9.8 , ELF 9.8 to < 11.3 , and ≥ 11.3 , according to development of liver-related outcomes, is summarized in Table 2. Of the 19 patients with a liver-related complication, baseline ELF score was ≥ 11.3 in 5 (26.3%), 9.8 to < 11.3 in 9 (47.4%), and < 9.8 in 5 patients (26.3%).

Each patient's baseline scores according to baseline LSM and presence of liver-related complications are represented in Fig. 1, which illustrates the clustering of several high scores in patients with liver outcomes.

The relationship between liver histology and clinical outcomes was not assessed in our study because liver biopsy was performed in only a small number of patients ($n = 47$) at baseline.

LIVER-RELATED COMPLICATIONS AND SIMPLE FIBROSIS SCORES AT FOLLOW-UP

A follow-up NFS was not available for 21 patients (BMI > 1 year from corresponding blood test results, $n = 20$; myelodysplastic syndrome, $n = 1$). Six of the 7 patients with a liver decompensation event or primary liver cancer at follow-up also had a high-risk NFS at follow-up. One patient with a liver decompensation event (and ascites at recruitment) did not have a follow-up NFS due to the lack of a recorded BMI. Of the 18 patients with a follow-up NFS and any liver-related complication, NFS was high risk in 13 patients (18 liver-related complications) and indeterminate in 5 patients (5 with portal hypertension; Table 3).

TABLE 3. LIVER-RELATED OUTCOMES, NFS AND FIB-4 SCORE CATEGORIES

	Follow-Up NFS (*n = 222)			Follow-Up FIB-4 Score (†n = 242)			PValue
	Low	Indeterminate	High	Low	Indeterminate	High	
	n = 41	n = 110	n = 71	n = 184	n = 45	n = 13	
Follow-up time (years), median (IQR)	3.63 (3.36-4.07)	4.24 (3.84-4.58)	4.28 (3.74-4.49)	4.15 (3.70-4.47)	4.34 (4.04-4.57)	4.11 (3.11-4.39)	0.056‡
Liver-related complications§							
Primary liver cancers, n (%)	0	0	4 (5.8%)	0	1 (2.3%)	3 (25.0%)	<0.001
HCC, n (%)	0	0	3 (4.2%)	0	0	3 (23.1%)	<0.001
Ascites, n (%)¶	0	0	1 (1.4%)	1 (0.5%)	0	1 (7.7%)	0.14
Varices, n (%)	0	3 (2.7%)	10 (14.1%)	3 (1.6%)	4 (8.9%)	6 (46.2%)	<0.001
Variceal bleed, n (%)	0	0	1 (1.4%)	0	0	1 (7.7%)	0.054
Hepatic encephalopathy, n (%)	0	0	1 (1.4%)	0	0	1 (7.7%)	0.054
Portal hypertension, n (%)¶	0	5 (4.5%)	12 (16.9%)	7 (3.8%)	4 (8.9%)	7 (53.8%)	<0.001
Pairwise comparison of NFS and FIB-4 score categories at baseline and follow-up							
Baseline NFS, n (row %)							
Low, n = 39	31 (79.5%)	8 (20.5%)	0				0.161*
Indeterminate, n = 124	10 (8.1%)	87 (70.2%)	27 (21.8%)				
High, n = 59	0	15 (25.4%)	44 (74.6%)				
Baseline FIB-4 score, n (row %)							
Low, n = 143				123 (86.0%)	18 (12.6%)	2 (1.4%)	<0.001#
Indeterminate, n = 89				57 (64.0%)	25 (28.1%)	7 (7.9%)	
High, n = 10				4 (40.0%)	2 (20.0%)	4 (40.0%)	

*n = 222 (n = 20 excluded as BMI > 1 year from corresponding blood test results; n = 1 excluded with myelodysplastic syndrome).

†n = 242 (n = 1 patient excluded due to myelodysplastic syndrome).

‡Kruskal-Wallis test.

§patients may have more than one liver-related outcome; liver-related complications include decompensation events (hepatic encephalopathy, ascites, variceal hemorrhage), primary liver cancer (HCC and cholangiocarcinoma), and portal hypertension (splenomegaly, varices or portosystemic collaterals).

||Fisher's exact test.

¶follow-up NFS unavailable in 1 patient (BMI > 1 year from corresponding blood test results).

#Broker's test.

Abbreviations: DE, decompensation events; PLC, primary liver cancer.

A follow-up FIB-4 score was not available for 1 patient with myelodysplasia. Five of the 7 patients with a liver decompensation event or primary liver cancer had a high-risk FIB-4 at follow-up (1 patient with cholangiocarcinoma had an indeterminate score and 1 patient with ascites had a low score). Of the 19 patients with any liver-related complication, FIB-4 was high risk in 7 patients (12 liver-related complications), indeterminate in 5 patients (5 liver-related complications), and low risk in 7 patients (7 liver-related complications).

As illustrated in Table 3, when comparing baseline NFS with follow-up scores, there was no statistically significant change in NFS categories ($P = 0.161$). Regarding FIB-4, most patients with a low FIB-4 at baseline had a low score at follow-up (86%); 64% of patients with an intermediate score at baseline had a low score at follow-up; of the small number of high scores at baseline ($n = 10$), 60% had an intermediate or low score at follow-up ($P < 0.001$).

NON-LIVER MALIGNANCY AND CARDIOVASCULAR EVENTS ACCORDING TO BASELINE LSM, ELF, AND SIMPLE FIBROSIS SCORE CATEGORIES

The patterns of development of malignancy (excluding nonmelanoma skin cancer) and cardiovascular events, according to baseline LSM, simple fibrosis scores, and ELF score are shown in Supporting Table S3. There was no significant difference in malignancy events according to baseline LSM, ELF, NFS, and FIB-4 categories and cardiovascular events according to baseline LSM and ELF categories. There was a higher proportion of cardiovascular events in patients with indeterminate and high-baseline NFS categories ($P < 0.001$). In contrast, there was a higher proportion of cardiovascular events in patients with indeterminate and low-baseline FIB-4 categories ($P = 0.033$). In Cox regression analysis of the abovementioned associations, no HR was statistically significant (all $P > 0.113$).

Discussion

Noninvasive tests of liver disease severity are recommended for use in community and primary care settings to exclude advanced fibrosis and to select

people requiring further assessment for high-risk NAFLD. There is limited information, however, about how well these tests identify the subgroup of people with NAFLD who develop liver- or non-liver-related clinical events. In this study, we assessed whether individual simple fibrosis scores, LSM, or serum ELF test predicted adverse clinical outcomes in an at-risk population that was assessed for NAFLD with clinically significant fibrosis. During a median follow-up of 50 months, decompensated liver disease or primary liver cancer occurred in 6 of 35 (17.1%) patients with baseline LSM > 13 kPa, 1 of 17 (5.9%) patients with LSM 9.5-13 kPa, and in no patients with LSM < 9.5 kPa. In 4 of the 7 patients who developed decompensation or primary liver cancer, NAFLD with clinically significant fibrosis was a new diagnosis at the time of initial recruitment and assessment of this high-risk population.

This study demonstrates that, using current LSM cut-off values for advanced fibrosis (≥ 9.5 kPa) and cirrhosis (> 13 kPa), LSM can identify the subgroup of patients with NAFLD at risk of future liver-related decompensation events and mortality as well as patients at risk of developing portal hypertension, including gastroesophageal varices. These findings support an earlier study of consecutive patients with NAFLD ($n = 2,245$) presenting to two centers for noninvasive diagnosis of liver fibrosis in France and Hong Kong.⁽¹⁸⁾ Over a median follow-up of 27 months, baseline LSM > 12 kPa was an independent predictor of overall survival (adjusted HR, 2.85; 95% CI, 1.65-4.92; $P = 0.0002$) and liver events (Gray test, $P < 0.0001$) and the incidence of HCC increased with baseline LSM (< 12 kPa, 0.32%; 12-18 kPa, 0.58%; 18-38 kPa, 9.26%; and > 38 kPa, 13.3%).⁽¹⁸⁾ Similarly, in a cohort of patients with NAFLD and compensated advanced chronic liver disease ($n = 1,039$) followed for a median time of 36 months, baseline LSM predicted liver decompensation (HR, 1.03; 95% CI, 1.02-1.04; $P < 0.001$) and liver-related death (HR, 1.02; 95% CI, 1.02-1.03; $P = 0.005$).⁽¹⁹⁾ It is of interest that none of the noninvasive tests studied were informative for cardiovascular or non-liver cancer events. Our data agree with results reported in the recent large study of NAFLD and compensated advanced chronic liver disease in which baseline LSM (HR, 1.01; 95% CI, 0.99-1.03; $P = 0.15$) was not associated with occurrence of cardiovascular events at univariate Cox regression analysis.⁽¹⁹⁾ In the latter study, baseline

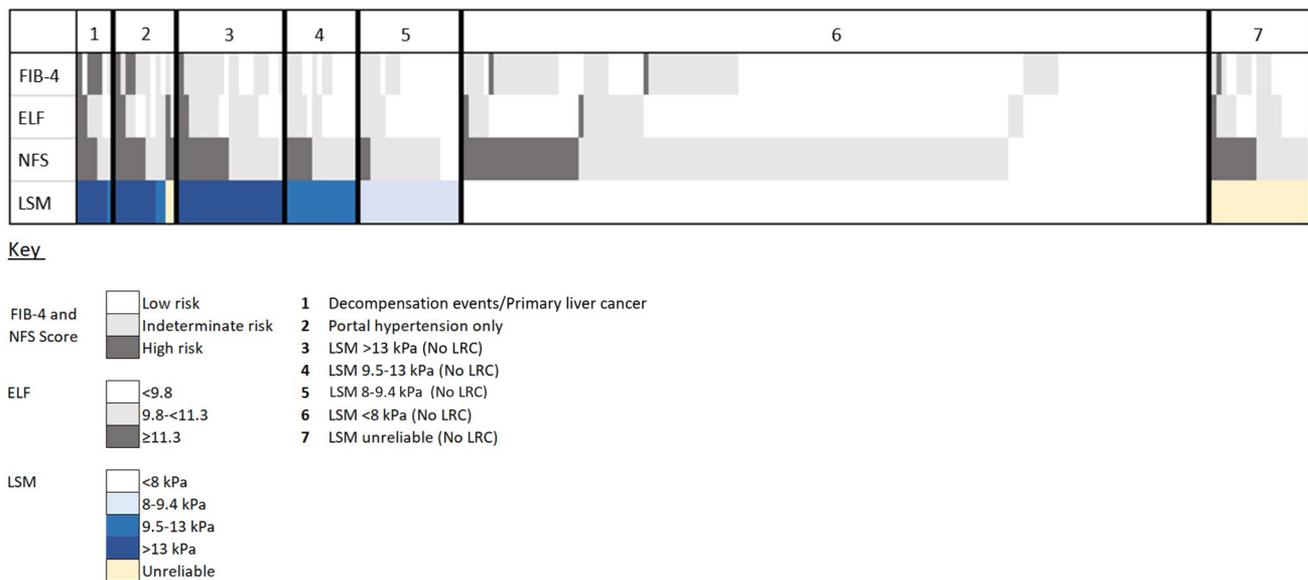


FIG. 1. Heatmap illustrating baseline scores according to LSM and liver-related complications. Patients' baseline fibrosis scores were taken and follow-up data collected within 0.52 to 5.62 years. Patients who had a liver decompensation event and/or a diagnosis of primary liver cancer are shown in Section 1. All patients had an LSM ≥ 9.5 kPa and an NFS risk that was high or indeterminate. FIB-4 and ELF scores had low-risk values. Similar results were found for patients who had only portal hypertension. Abbreviations: DE, decompensation events; PLC, primary liver cancer; LRC, liver-related complications.

LSM was associated with occurrence of extrahepatic neoplasm (HR, 1.02; 95% CI, 1.00-1.04; $P = 0.03$) in the univariate analysis but not in the multivariate analysis (HR, 1.02; 95% CI, 0.99-1.04; $P = 0.12$).⁽¹⁹⁾ In a cohort of consecutive patients with NAFLD of any severity, patients with baseline LSM > 12 kPa had higher incidence of cardiovascular events at 1, 3, and 5 years than patients with LSM ≤ 12 kPa (Gray test, $P = 0.0004$), but LSM was not associated with incidence of non-liver cancer (Gray test, $P = 0.169$).⁽¹⁸⁾

Most primary care clinicians do not have ready access to FibroScan, and LSM is usually only obtained on referral to secondary care. However, simple scoring systems (FIB-4, NFS) are readily determined in primary care and demonstrate acceptable diagnostic performance for excluding advanced fibrosis and identifying patients requiring further assessment for high-risk NAFLD.⁽²⁰⁾ Importantly, our current study shows that NFS can also identify the subgroup of patients with NAFLD at low risk of future liver-related clinical events. In particular, no patient with low-risk NFS developed liver decompensation or liver-related mortality. Following repeat NFS at the end of follow-up, all patients with a liver-related complication were in the high-risk NFS category, suggesting that

longitudinal changes in the NFS score⁽²¹⁾ may have a role in predicting adverse liver outcomes.

Patients who developed liver-related complications were also more likely to have baseline high-risk FIB-4 scores or ELF test ≥ 9.8 compared to patients who did not develop liver-related events. However, in this cohort, a low-risk FIB-4 score or ELF test < 9.8 did not preclude the development of liver-related complications. Our study was not designed to compare the predictive value of the noninvasive tests, and it is likely that a combination of these tests may be better predictors of liver-related outcomes in different situations. Our findings support and extend an earlier retrospective study of patients with NAFLD ($n = 320$) with a median follow-up period of 104.8 months in which both intermediate- and high-risk NFS categories at baseline were significantly associated with a higher likelihood to develop liver-related events.⁽²²⁾ In the latter study, only the high-risk FIB-4 category increased the likelihood of developing liver-related events compared to the low-risk category.⁽²²⁾

Our study affirms the high prevalence of cardiovascular disease present in more than one third of the cohort at follow-up and responsible for the most common cause of death. Extrahepatic malignancy was

also common (present in 14% of the cohort at follow-up) and was the second most common cause of death, along with liver-related complications. The rate of development of a decompensation event or HCC was low (2.1% per year) in our patients with compensated cirrhosis (LSM > 13 kPa) and is consistent with the annual rate of decompensation from baseline compensated cirrhosis (2.4%) in a large community-based study of NAFLD in the United States (n = 98,312 patients, followed for a median of 4.13 years).⁽²³⁾ However, due to the high prevalence of NAFLD in people with obesity and T2D, these events are projected to lead to a substantial increase in NAFLD-related disease burden over the next decade.⁽⁵⁾

Although our study lacked a formal protocol to follow patients without advanced fibrosis who were not reviewed in the hepatology clinic, we believe that if liver-related complications, cardiac events, or malignancy occurred, these events were likely to be recorded in the hospital and/or primary care electronic medical record. Key strengths of our study are the assessment of clinical outcomes in a cohort of patients from an endocrine clinic and primary care facility who were screened for NAFLD with clinically significant fibrosis and follow-up data were collected for all patients included in the study. Nevertheless, our findings should be interpreted with caution due to the small sample size and relatively short follow-up, particularly for some of the study endpoints (e.g., malignancy events) resulting in little statistical power to assess differences between groups with certainty. As a result, there may have been differences that the study did not detect. The data confirm that liver fibrosis risk stratification in non-hepatology settings can identify the subset of patients at risk of liver-related complications who require referral for specialist care. Nevertheless, further work is required in a larger cohort of patients and with longer follow-up data to assess whether this approach improves clinical outcomes and is cost effective by decreasing unnecessary hepatology referrals.

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REFERENCES

- 1) Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: from pathophysiology to therapeutics. *Metabolism* 2019;92:82-97.
- 2) Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in

- patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol* 2019;71:793-801.
- 3) Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwithaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389-397.e10.
- 4) Wong VW, Adams LA, de Ledinghen V, Wong GL, Sookoian S. Noninvasive biomarkers in NAFLD and NASH - current progress and future promise. *Nat Rev Gastroenterol Hepatol* 2018;15:461-478.
- 5) Adams LA, Roberts SK, Strasser SI, Mahady SE, Powell E, Estes C, et al. Nonalcoholic fatty liver disease burden: Australia, 2019-2030. *J Gastroenterol Hepatol* 2020;35:1628-1635.
- 6) Brain D, O'Beirne J, Hickman IJ, Powell EE, Valery PC, Kularatna S, et al. Protocol for a randomised trial testing a community fibrosis assessment service for patients with suspected non-alcoholic fatty liver disease: LOCAL assessment and triage evaluation of non-alcoholic fatty liver disease (LOCATE-NAFLD). *BMC Health Serv Res* 2020;20:335.
- 7) Chalmers J, Wilkes E, Harris R, Kent L, Kinra S, Aithal GP, et al. The development and implementation of a commissioned pathway for the identification and stratification of liver disease in the community. *Frontline Gastroenterol* 2020;11:86-92.
- 8) Davyduke T, Tandon P, Al-Karaghoul M, Abiralde JG, Ma MM. Impact of implementing a "FIB-4 first" strategy on a pathway for patients with NAFLD referred from primary care. *Hepatol Commun* 2019;3:1322-1333.
- 9) El-Gohary M, Moore M, Roderick P, Watkins E, Dash J, Reinson T, et al. Local care and treatment of liver disease (LOCATE) - A cluster-randomized feasibility study to discover, assess and manage early liver disease in primary care. *PLoS One* 2018;13:e0208798.
- 10) Srivastava A, Gailer R, Tanwar S, Trembling P, Parkes J, Rodger A, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol* 2019;71:371-378.
- 11) Hayward KL, McKillen BJ, Horsfall LU, McIvor C, Liew K, Sexton J, et al. Towards collaborative management of nonalcoholic fatty liver disease (TCM-NAFLD): a 'real-world' pathway for fibrosis risk assessment in primary care. *Intern Med J* 2021; <https://doi.org/10.1111/imj.15422>.
- 12) Eslam M, Sarin SK, Wong VW, Fan JG, Kawaguchi T, Ahn SH, et al. The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. *Hepatol Int* 2020;14:889-919.
- 13) Patel P, Hossain F, Horsfall LU, Banh X, Hayward KL, Williams S, et al. A pragmatic approach identifies a high rate of nonalcoholic fatty liver disease with advanced fibrosis in diabetes clinics and at-risk populations in primary care. *Hepatol Commun* 2018;2:893-905.
- 14) Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction* 1993;88:791-804.
- 15) McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. *Am J Gastroenterol* 2017;112:740-751.
- 16) Siemens Healthcare Diagnostics Inc. Literature Compendium Volume I: The Enhanced Liver Fibrosis (ELF) Blood Test. https://cdn0.scrvt.com/39b415fb07de4d9656c7b516d8e2d907/8f5cd5ed0014/98d5a628acad/30-21-DX-1022-76_ELFLiteratureCompendium_Vol1_Rev04-V4.pdf. Accessed July 2021.

- 17) Day J, Patel P, Parkes J, Rosenberg W. Derivation and performance of standardized enhanced liver fibrosis (ELF) test thresholds for the detection and prognosis of liver fibrosis. *J Appl Lab Med* 2019;3:815-826.
- 18) Shili-Masmoudi S, Wong GL, Hiriart JB, Liu K, Chermak F, Shu SS, et al. Liver stiffness measurement predicts long-term survival and complications in non-alcoholic fatty liver disease. *Liver Int* 2020;40:581-589.
- 19) Petta S, Sebastiani G, Vigano M, Ampuero J, Wong VW, Boursier J, et al. Monitoring occurrence of liver-related events and survival by transient elastography in patients with nonalcoholic fatty liver disease and compensated advanced chronic liver disease. *Clin Gastroenterol Hepatol* 2021;19:806-815.e5.
- 20) McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010;59:1265-1269.
- 21) Patel PJ, Cheng JC, Banh X, Gracen L, Radford-Smith D, Hossain F, et al. Clinically significant fibrosis is associated with longitudinal increases in fibrosis-4 and nonalcoholic fatty liver disease fibrosis scores. *Clin Gastroenterol Hepatol* 2020;18:710-718.e4.
- 22) Angulo P, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013;145:782-789.e4.
- 23) Nyberg LM, Cheetham TC, Patton HM, Yang SJ, Chiang KM, Caparosa SL, et al. The natural history of NAFLD, a community-based study at a large health care delivery system in the United States. *Hepatol Commun* 2020;5:83-96.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1852/supinfo.